Massachusetts Department of Public Health

State Laboratory Institute

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SLI Selected to Participate in Evaluation of Riboprinter®

by John Fontana

The State Laboratory Institute is one of five regional laboratories selected by the Centers for Disease Control and Prevention (CDC) to participate in the evaluation of the Qualicon Riboprinter®. The goal of the evaluation is to determine if the Riboprinter® can be used by state public health laboratories to enhance other DNA fingerprinting techniques and molecular methods used in outbreak investigations and species identification.

The Riboprinter® is an instrument that automates the process of ribotyping bacteria. Ribotyping is a DNA fingerprinting technique that involves probing the DNA of a cell with known DNA sequences from ribosomal RNA genes, hence the term "ribo" typing. Bacterial chromosomal DNA is cut into many fragments by a restriction enzyme, sorted by size in an agarose gel by electrophoresis, then probed with labeled DNA. Only fragments that contain sequences complementary to the probe DNA will be labeled. After addition of an enzyme-antibody conjugate, which binds to the label, and a colorless substrate, which is converted to a blue color by the enzyme, the labeled DNA appears as a band. There are usually between 7 and 12 bands for each isolate.

Ribosomal RNA genes are highly conserved, so different bacterial species have similar rRNA genes. However, there are several copies of rRNA genes in each cell, and their number and specific location on the chromosome varies among species. Therefore, banding patterns of the chromosomal DNA that are hybridized by the RNA gene probe can reflect differences at the species and subspecies level.

Manual ribotyping consists of separate laboratory procedures: cell lysis, deproteination, DNA restriction, electrophoresis, DNA transfer to membrane, hybridization and detection of bound DNA probes. This typically takes at least 24 hours to complete. As with any multi-step procedure, there is the potential for technical errors. The Riboprinter® automates these procedures and provides final results in eight hours, including image capture and analysis of the ribotype pattern. In addition to the time savings, reproducibility of test results is improved.

For the next year, every fifth *Salmonella* spp. and *Campylobacter* spp., and all *Listeria monocytogenes* that are received by the Enteric and Reference Laboratories will be analyzed by ribotyping. Ribotype patterns from these isolates will be compared to a library of over 1200 ribotype patterns of known bacterial species and assigned a pattern designation that groups the isolate into a species level, or in some cases, a sub-species level ribotype. In the event that a pattern does not match an existing pattern in the database, a new ribotype pattern is created and added to the database after confirmation.

The ribotypes of the isolates used in the evaluation will be compared to 1) serotypes and PFGE patterns of *Salmonella* spp., 2) PFGE patterns of *Listeria monocytogenes*, and 3) other typing methods of *Campylobacter* spp. Our evaluation will focus on the ability of the Riboprinter® to rapidly identify species and to discriminate between isolates at the subspecies level. This evaluation is an example of the State Laboratory Institute's interest in assessing the latest technology to support investigations done by the state and federal communicable disease control programs. For additional information contact Dr. John Fontana by e-mail at john.fontana@state.ma.us or the PFGE laboratory at 617-983-6619.

Joint Educational Initiative Includes Public Health in MLS Curriculum

by Marcia Stowell and Garry Greer

The State Laboratory Institute (SLI) and the Medical Laboratory Science (MLS) Department at the University of Massachusetts-Dartmouth have added a unique component to the Clinical Laboratory Science (CLS) program that will give senior level students a didactic and practical introduction to the public health laboratory.

Traditionally, CLS program curricula consist of either a four-year university program that includes a one-year hospital component (3+1 program) or a four-year university program in a laboratory-related science plus a one-year hospital program. The hospital component includes rotations through the clinical laboratories for practical experience. Several variations of the above models exist, such as Northeastern University's five-year program that includes

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both hospital rotations and cooperative work experience. Currently there are four NAACLS accredited university / college based CLS Programs in Massachusetts.

The University of Massachusetts at Dartmouth has a four-year CLS program, in which the students begin practical clinical laboratory courses in their freshmen year. Modern laboratories at the university have instrumentation that is basic to all clinical laboratories. Laboratory theory and methods are integrated throughout the program, which culminates with clinical practica at affiliated hospitals; students rotate through hematology, chemistry, immunology / blood banking, and microbiology. A newly added practicum at the State Laboratory Institute will enhance this program, to serve as a foundation and future resource and to provide valuable contacts and insight into disease prevention, intervention and control in the public health setting.

The structure of the SLI component consists of a one-day group orientation in the fall of the senior year, followed by a one-week practicum completed by each student. The orientation covers an overview of public health and public health laboratories, outlining the goals, infrastructure and interactions among government and state public health agencies, private health care facilities and the public. Students are able to meet with and ask questions of the Bureau and Program Directors and Supervisors at SLI for both Laboratory Sciences and Communicable Disease Control, including epidemiology. Tours and presentations cover programs and laboratory testing

UMASS-Dartmouth Clinical Laboratory Science students and faculty with SLI staff during first orientation.

performed at SLI. Students also review a collection of public health journals and reference texts and receive a list of resources related to the public health laboratory.

The laboratory practica allow students to work one-on-one with laboratory specialists at SLI. With the aid of their university advisors, students select an area of interest for their practica, choosing from a number of laboratory areas that perform tests not typically available in most hospital laboratories. Principles of testing, quality assurance, safety and confidentiality are combined with observation and hands-on, supervised practice of laboratory analyses.

Because State laboratories form a bridge between federal public health agencies, such as the Centers for Disease Control and Prevention (CDC) in Atlanta, and community laboratories, it is important for MLS students to see this connection early in their careers. In order to meet the challenges of public health, state laboratories must communicate and work cooperatively with clinical laboratories in other settings. Making public health an integral component of MLS education benefits students as well as the field of public health.

Analysis of Biogenic Amines Associated with Scombroid Poisoning

by Lorna Bosworth

Scombroid poisoning is a chemical intoxication resulting from the ingestion of fish containing the biogenic amines, histamine, putrescine and cadaverine. These compounds are the breakdown products of naturally occurring amino acids in fish. Decomposition of fish tissue by bacteria results in the decarboxylation of the amino acids producing an accumulation of biogenic amines (Figure 1). This decomposition

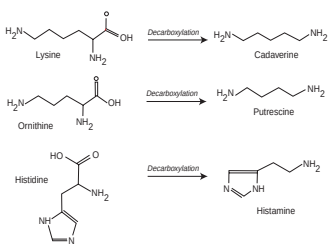

Figure 1 Amino Acid Decarboxylation Products

occurs after prolonged exposure to ambient temperature and is accelerated by high temperatures. Decomposition can occur from improper handling of fish during harvesting, cleaning and storage. Scombroid poisoning most often is associated with fish of the family *Scombridae*, which includes tuna, mackerel and bonito. However, fish species from other families such as bluefish or mahi mahi have been implicated in scombroid poisoning.

Scombroid poisoning is characterized by a relatively rapid onset of illness, often as short as 30 minutes. Symptoms resemble an allergic reaction and include hives, headache, nausea, vomiting, diarrhea, and hypertension. Affected individuals usually respond well to treatment with antihistamines.

The State Laboratory Institute analyzes fish implicated in scombroid poisoning using a method developed at the University of Barcelona (Veciana-Nogues *et al*, 1995 JAOAC 78,4). Homogenized fish tissue is extracted multiple times with trichloroacetic acid, centrifuged and the supernatant fluid filtered. The extract is analyzed by gradient elution, reverse phase HPLC with post column derivatization and fluorescence detection (excitation wavelength=340nm, emission wavelength=445nm). Figure 2 shows a chromatogram of a standard containing biogenic amines.

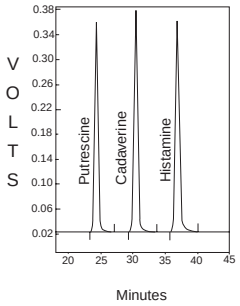

Figure 2. Chromatogram of Biogenic Amine Standard

The US FDA has established an action level of 500 ppm of histamine in fish, a concentration at which some individuals experience adverse health effects. There are no established safety limits for putrescine and cadaverine. However, they are useful indicators of decomposition and there is some evidence that they act synergistically with histamine to increase the severity of human illness.

For additional information or to report suspected cases of scombroid poisoning, please contact the Massachusetts Division of Food & Drugs at 617-983-6712.

Grants, Projects & Publications —

Spoligotyping Grant Received by SLI

by Alex Sloutsky

DNA fingerprinting of *Mycobacterium tuberculosis* isolates has proven to be a valuable tool to support the investigation of outbreaks and to assist in the clarification of suspected cross-contamination or mislabeling. The Massachusetts TB Prevention and Control Division and the State Laboratory Institute's TB Laboratory currently participate in the National TB Genotyping and Surveillance Network project. This project was designed to evaluate the utility of large scale strain typing and DNA fingerprint cluster identification to TB control programs. These activities have relied upon the international standard restriction fragment length polymorphism (RFLP) method based on IS6110, which provides highly specific fingerprint patterns.

However, technical complexity, combined with the requirement for fully grown cultures of *M. tuberculosis* for DNA preparation, makes RFLP less than ideal as a routine screening method for rapid assessment of strain relatedness. For this reason the Federal Centers for Disease Control and Prevention (CDC) has funded the TB Laboratory and the TB Control Division to participate in a multi-center study of spoligotyping as an alternative to RFLP typing.

Spoligotyping (spacers oligonucleotide typing) is a simpler, more rapid, though less specific subtyping method. Since it is a PCR-based technique, it can be applied to small numbers of tubercle bacilli, such as concentrated sediments from AFB smear-positive clinical specimens. Thus, this method may allow routine, timely monitoring of all *M. tuberculosis* isolates to detect or rule-out potential community transmission and to "fast-track" possible specimen cross-contamination. In a short period of time, spoligotyping has been implemented in the TB Laboratory, and the actual cluster investigations by the TB Control staff are about to begin.

For further information about this or related TB projects please contact Dr. Alexander Sloutsky by e-mail: alex.sloutsky@state.ma.us or by phone: 617-983-6370.

EID Fellow Completes Public Health Laboratory Training at SLI

by Marcia Stowell

The Emerging Infectious Disease (EID) Advanced Laboratory Training Fellowship was recently completed at SLI by Eyob Mazengia. Eyob came to the State Laboratory Institute having completed his Master of Science in Public Health at the University of Washington, in Seattle. During his one-year fellowship program at SLI, he worked with the Director of the Diagnostic Laboratories, Dr. Harvey George, and with laboratory specialists in the area of respiratory pathogens.

Eyob began his fellowship by reviewing all routine procedures for isolation and identification of enteric and respiratory pathogens, and then by gaining expertise in the more specialized molecular procedures. He then undertook a specialized study to describe the recovery of *B. holmesii* from nasopharyngeal specimens, and also to report on the use of PFGE as an epidemiological tool for determining genotypic relatedness among cases of *B. holmesii*. (See SLI Newsletter, July 1999, Vol. 1, No. 7, p. 2.) He presented an original abstract at the 1999 General Meeting of the American Society for Microbiology. A manuscript of his complete work, entitled "Recovery of *Bordetella holmesii* from Patients with Pertussis-Like symptoms: Use of Pulsed-Field Gel Electrophoresis to Characterize Circulating Strains" has been accepted for publication to the Journal of Clinical Microbiology.

In addition to his specific projects under the EID Fellowship, Eyob observed and trained in other areas of the laboratory at SLI in order to gain knowledge of specialized procedures and to better understand the interactions between the laboratory and epidemiology staff.

Eyob Mazengia (center), EID Training Fellow 1999, with Daniel Rowe and Deborah Shea of the SLI PFGE laboratory.

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Laboratory Training Activities

Expert Witness Workshop for the Laboratorian - State Laboratory Institute, Boston, MA, June 5: Full-day workshop. Fee $75. Call (617) 983-6285.

Public Health Teleconference Series - State Laboratory Institute, Boston, MA: The Impact of Rapid Testing on the Laboratory and Surveillance, Oct. 3; Antibiotic Resistance Surveillance, Nov. 14. Fee $35 per site per program. Call (617) 983-6285.

State Laboratory Training Coordinator, *Garry R. Greer, BS, (617) 983-6608, E-mail: garry.greer@state.ma.us.*
For a list of NLTN courses in your area sign on to the Web at http://www.cdc.gov/phppo/dls/nltn.htm.

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